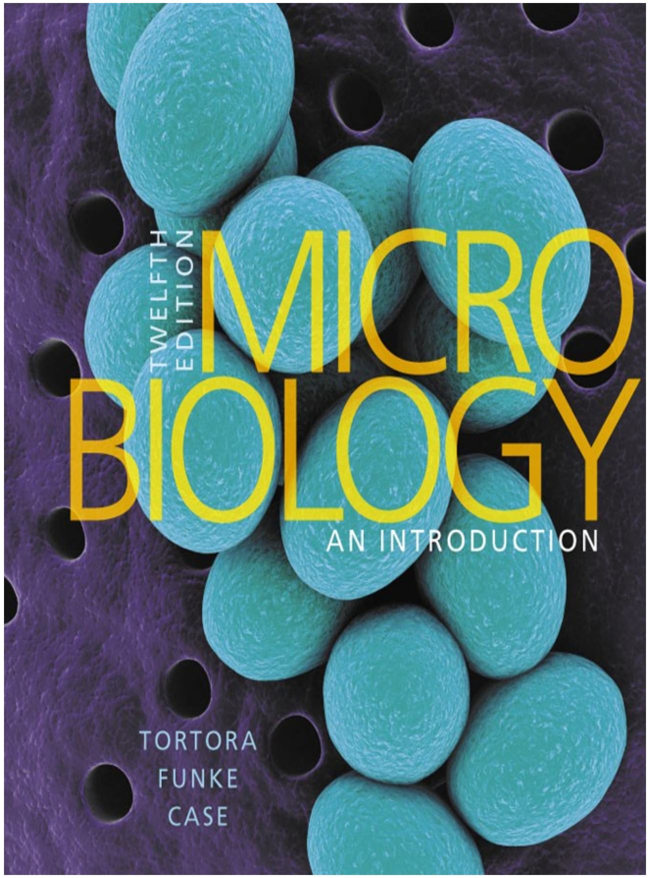
Microbiology an Introduction

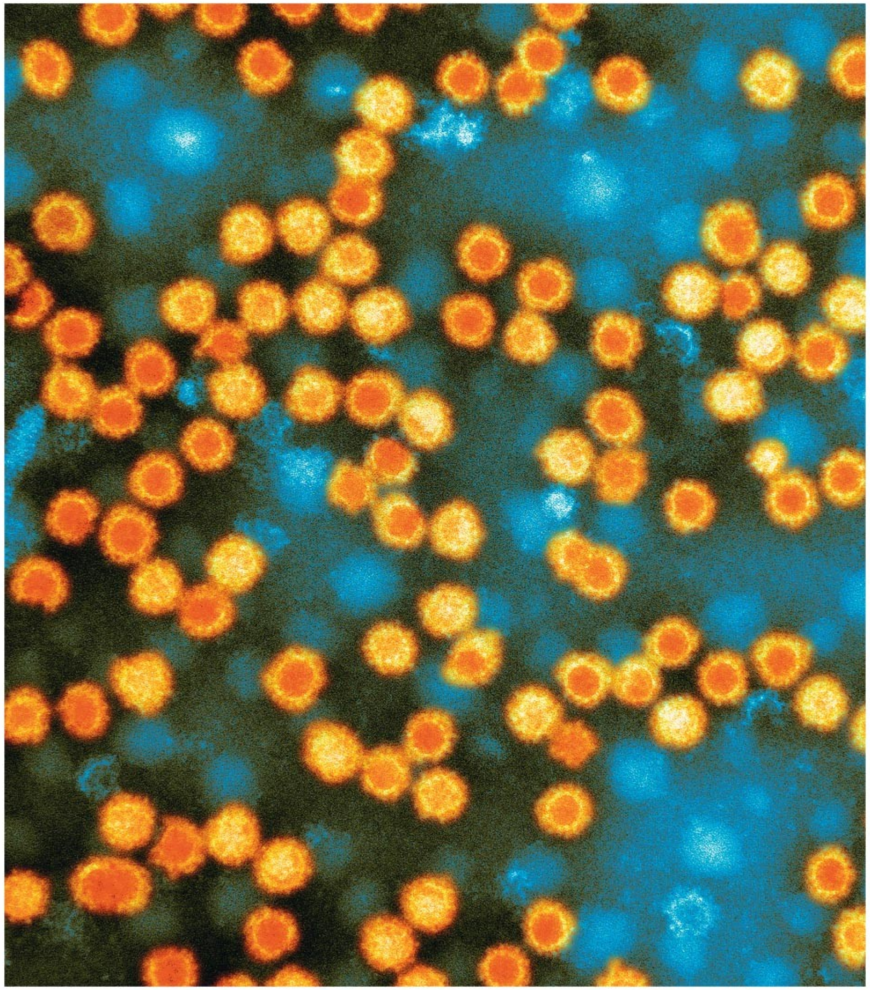
Twelfth Edition



Chapter 7

The Control of Microbial Growth

Norovirus



The Terminology of Microbial Control (1 of 4)

Learning Objective

7-1 Define the following key terms related to microbial control: **sterilization, disinfection, antisepsis, degerming, sanitization, biocide, germicide, bacteriostasis,** and **asepsis.**

The Terminology of Microbial Control (2 of 4)

- **Sepsis** refers to bacterial contamination
- **Asepsis** is the absence of significant contamination
  - Aseptic surgery techniques prevent the microbial contamination of wounds

The Terminology of Microbial Control (3 of 4)

- **Sterilization:** removing and destroying all microbial life
- **Commercial sterilization:** killing ***C. botulinum*** endospores from canned goods
- **Disinfection:** destroying harmful microorganisms
- **Antisepsis:** destroying harmful microorganisms from living tissue

The Terminology of Microbial Control (4 of 4)

- **Degerming:** the mechanical removal of microbes from a limited area
- **Sanitization:** lowering microbial counts on eating utensils to safe levels
- **Biocide (germicide):** treatments that kill microbes
- **Bacteriostasis:** inhibiting, not killing, microbes

Check Your Understanding-1

Check Your Understanding

- ✓ The usual definition of **sterilization** is the removal or destruction of all forms of microbial life; how could there be practical exceptions to this simple definition?
  7-1

The Rate of Microbial Death (1 of 3)

Learning Objective

7-2 Describe the patterns of microbial death caused by treatments with microbial control agents.

The Rate of Microbial Death (2 of 3)

Table 7.2 Microbial Exponential Death Rate: An Example

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

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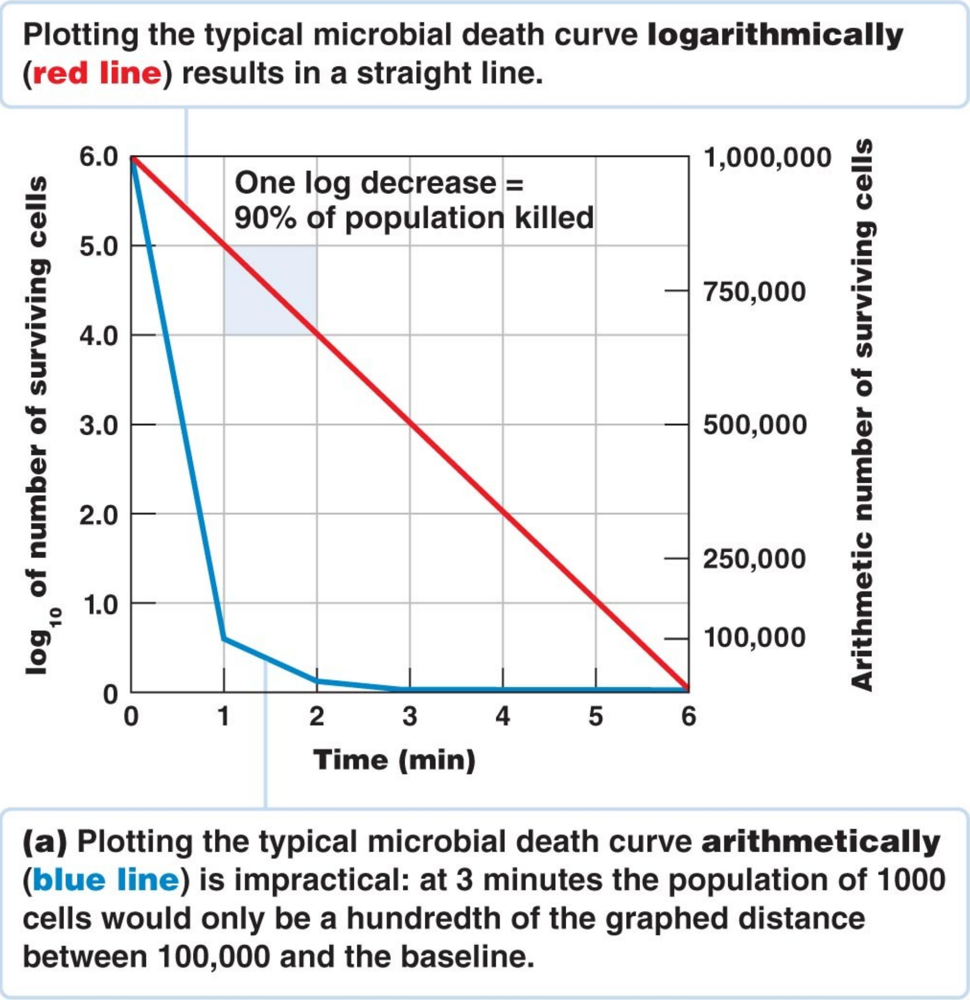
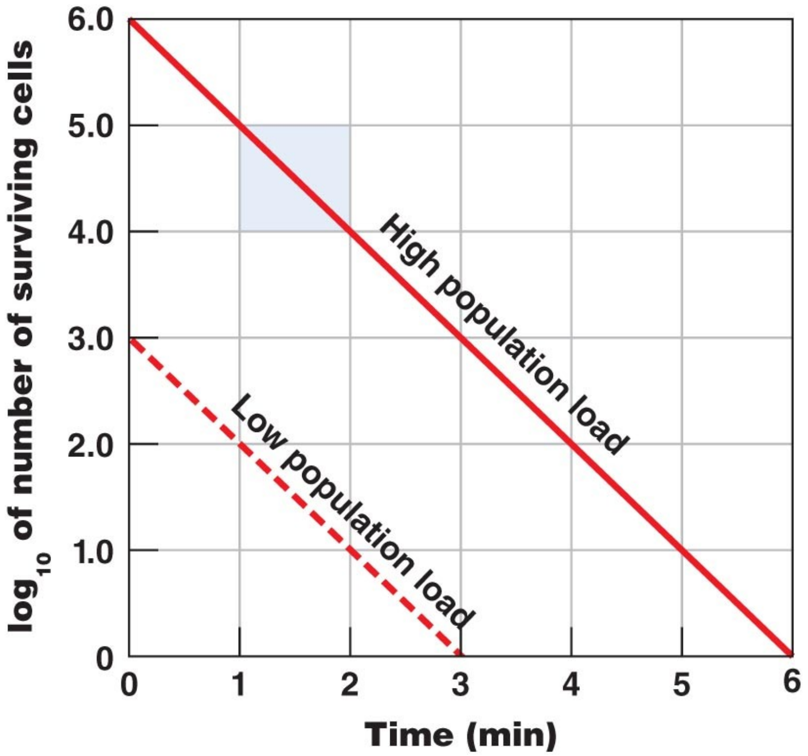
Figure 7.1a Understanding the Microbial Death Curve



The Rate of Microbial Death (3 of 3)

- Effectiveness of treatment depends on:
  - Number of microbes
  - Environment (organic matter, temperature, biofilms)
  - Time of exposure
  - Microbial characteristics

Figure 7.1b Understanding the Microbial Death Curve



(b) Logarithmic plotting (**red**) reveals that if the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one, whether using heat or chemical treatments.

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Check Your Understanding-2

Check Your Understanding

- How is it possible that a solution containing a million bacteria would take longer to sterilize than one containing a half-million bacteria?
  7-2

Actions of Microbial Control Agents (1 of 2)

Learning Objective

7-3 Describe the effects of microbial control agents on cellular structures.

Actions of Microbial Control Agents (2 of 2)

- Alteration of membrane permeability
- Damage to proteins (enzymes)
- Damage to nucleic acids

Check Your Understanding-3

Check Your Understanding

- ✓ Would a chemical microbial control agent that affects plasma membranes affect humans? 7-3

Physical Methods of Microbial Control (1 of 2)

Learning Objectives

7-4 Compare the effectiveness of moist heat (boiling, autoclaving, pasteurization) and dry heat.

7-5 Describe how filtration, low temperatures, high pressure, desiccation, and osmotic pressure suppress microbial growth.

7-6 Explain how radiation kills cells.

Heat (1 of 3)

- Heat denatures enzymes
- **Thermal death point (TDP):** lowest temperature at which all cells in a liquid culture are killed in 10 min
- **Thermal death time (TDT):** minimal time for all bacteria in a liquid culture to be killed at a particular temperature

Heat (2 of 3)

- **Decimal reduction time (DRT)**
  - Minutes to kill 90% of a population at a given temperature

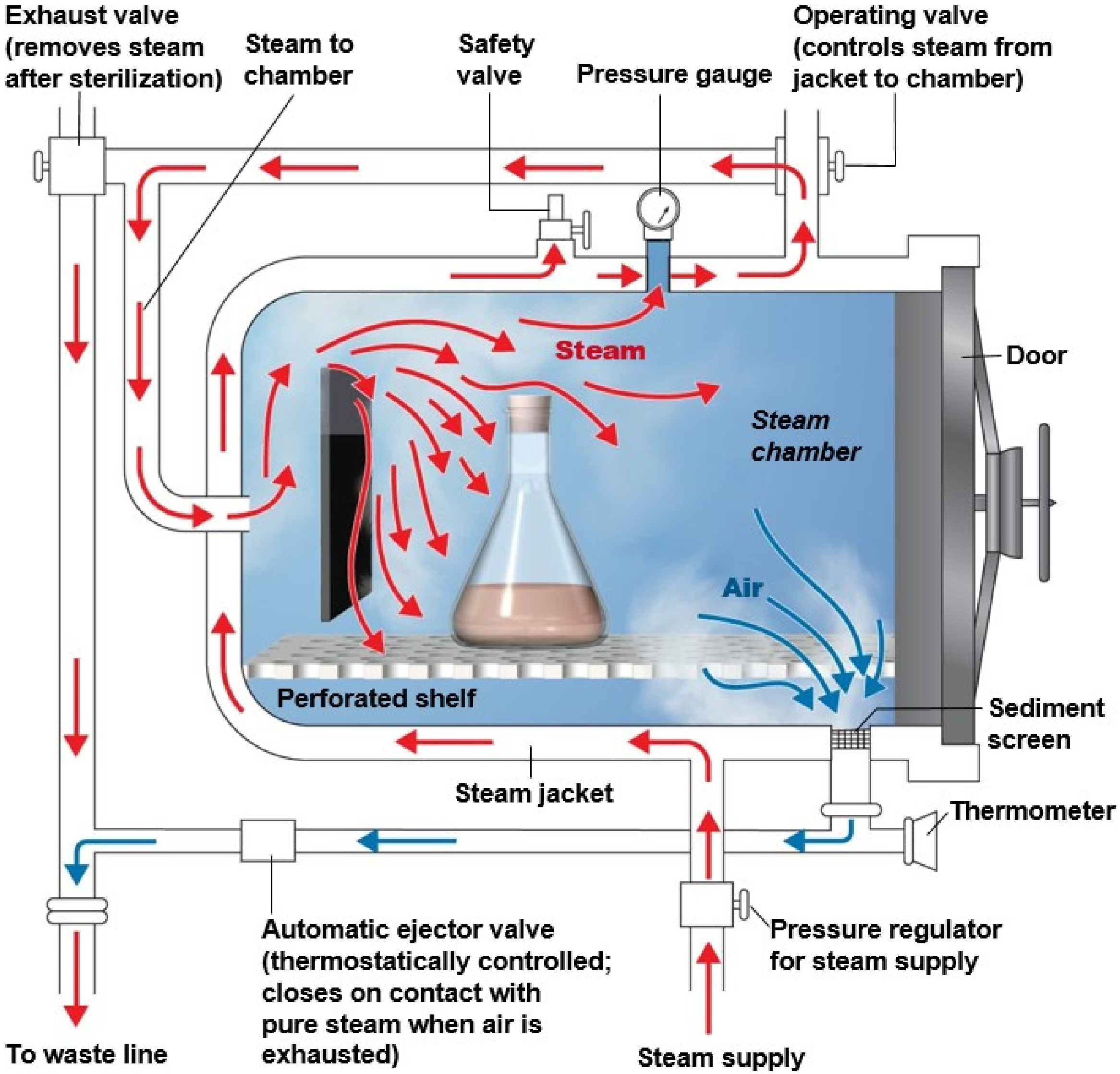
Moist Heat Sterilization (1 of 4)

- Moist heat denatures proteins
- Boiling
- Free-flowing steam

Moist Heat Sterilization (2 of 4)

- **Autoclave:** steam under pressure
- 121 C at 15 psi for 15 min
- Kills all organisms and endospores
- Steam must contact the item's surface

Figure 7.2 An Autoclave



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Moist Heat Sterilization (3 of 4)

- Large containers require longer sterilization times
- Test strips are used to indicate sterility

Moist Heat Sterilization (4 of 4)

Table 7.4 The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions*

Pressure (psi in Excess of Atmospheric Pressure)	Temperature (°C)
0	100
5	110
10	116
15	121
20	126
30	135

*At higher altitudes, the atmospheric pressure is less, a phenomenon that must be taken into account in operating an autoclave. For example, to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

Figure 7.3 Examples of Sterilization Indicators

Heat (3 of 3)

- **Pasteurization** reduces spoilage organisms and pathogens
- Equivalent treatments
  - 63°C for 30 min
  - **High-temperature short-time (HTST):** 72°C for 15 sec
  - **Ultra-high-temperature (UHT):** 140°C for 4 sec
- **Thermoduric** organisms survive

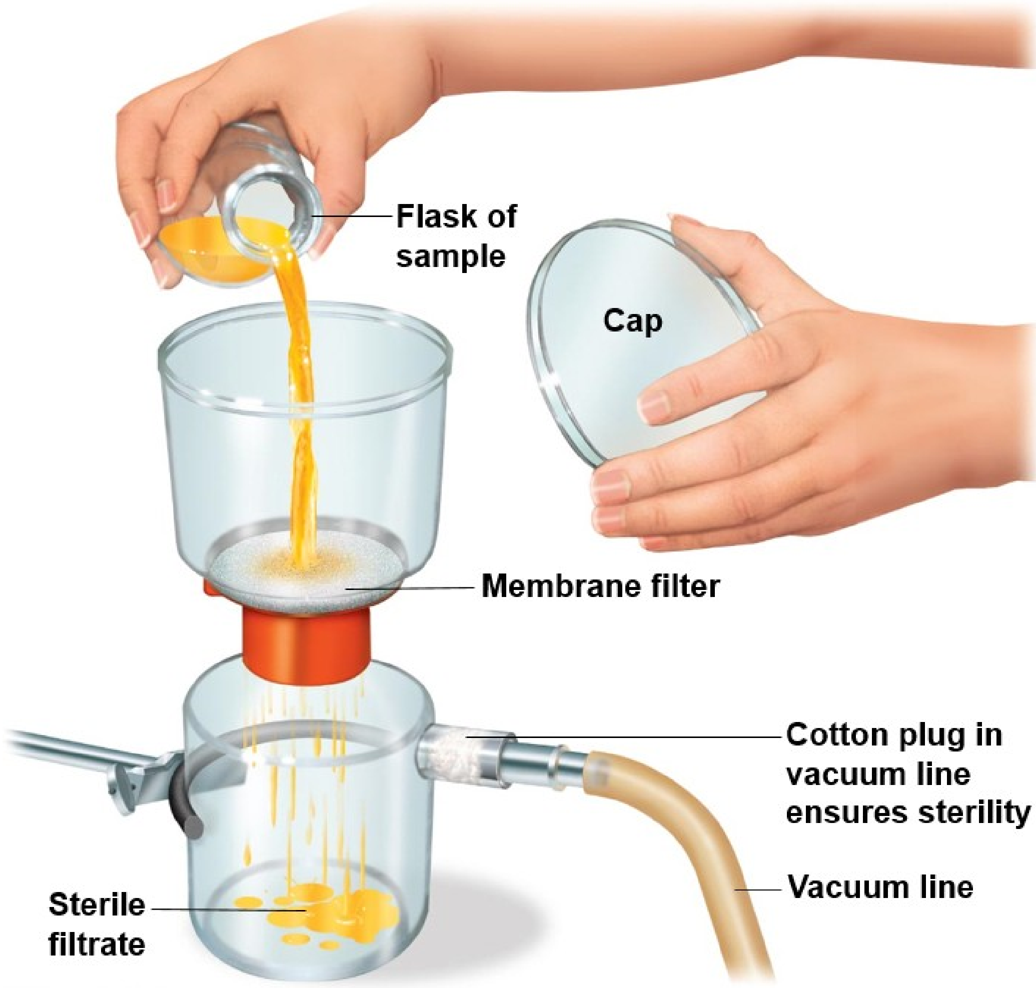
Dry Heat Sterilization

- Kills by oxidation
  - **Flaming**
  - Incineration
  - **Hot-air sterilization**

Filtration

- Passage of substance through a screenlike material
- Used for heat-sensitive materials
- **High-efficiency particulate air (HEPA) filters** remove microbes > 0.3 µm
- **Membrane filters** remove microbes > 0.22 µm

Figure 7.4 Filter Sterilization with a Disposable, Presterilized Plastic Unit



Physical Methods of Microbial Control (2 of 2)

- Low temperature has a bacteriostatic effect
  - Refrigeration
  - Deep-freezing
  - Lyophilization (freeze drying)
- High pressure denatures proteins
- **Desiccation**: absence of water prevents metabolism
- Osmotic pressure uses salts and sugars to create hypertonic environment; causes plasmolysis

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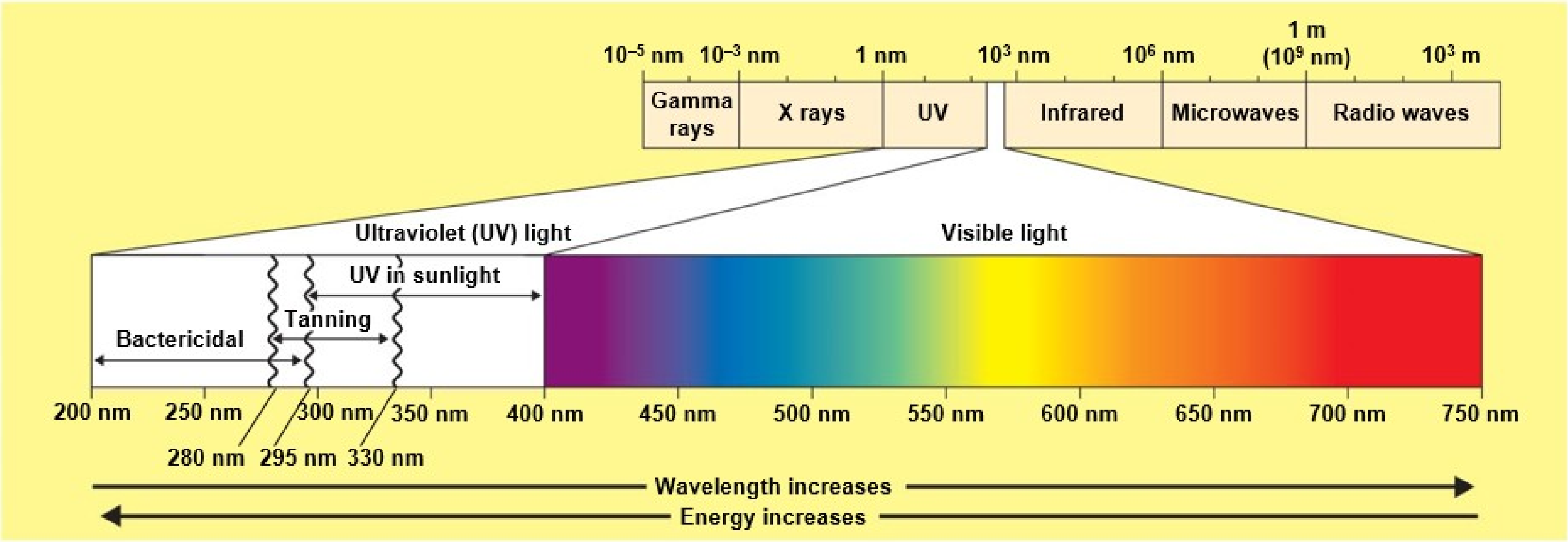
Radiation (1 of 2)

- **Ionizing radiation** (X rays, gamma rays, electron beams)
  - Ionizes water to create reactive hydroxyl radicals
  - Damages DNA by causing lethal mutations
- **Nonionizing radiation** (UV, 260 nm)
  - Damages DNA by creating thymine dimers
- **Microwaves** kill by heat; not especially antimicrobial

Figure 7.5 The Radiant Energy Spectrum



Check Your Understanding-4

Check Your Understanding

- ✓ How is microbial growth in canned foods prevented?
  7-4
- ✓ Why would a can of pork take longer to sterilize at a given temperature than a can of soup that also contained pieces of pork?
  7-5
- ✓ What is the connection between the killing effect of radiation and hydroxyl radical forms of oxygen?
  7-6

Chemical Methods of Microbial Control (1 of 2)

Learning Objectives

7-7 List the factors related to effective disinfection.

7-8 Interpret the results of use-dilution tests and the disk diffusion method.

Principles of Effective Disinfection

- Concentration of disinfectant
- Organic matter
- pH
- Time

Use-Dilution Tests

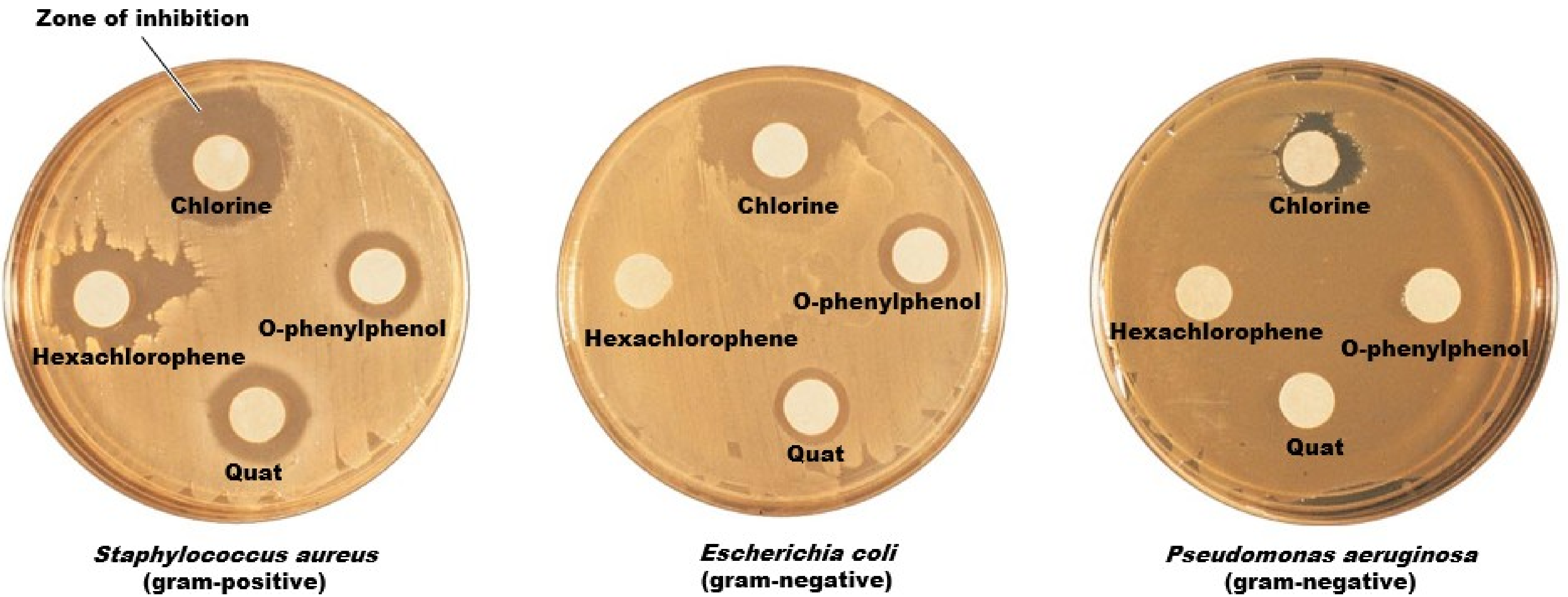
- Metal cylinders are dipped in test bacteria and dried
- Cylinders are placed in disinfectant for 10 min

  at 20°C
- Cylinders are transferred to culture media to determine whether the bacteria survived treatment

The Disk-Diffusion Method

- Evaluates efficacy of chemical agents
- Filter paper disks are soaked in a chemical and placed on a culture
- Look for zone of inhibition around disks

Figure 7.6 Evaluation of Disinfectants by the Disk-Diffusion Method



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Check Your Understanding-5

Check Your Understanding

- If you wanted to disinfect a surface contaminated by vomit and a surface contaminated by a sneeze, why would your choice of disinfectant make a difference?
  7-7
- Which is more likely to be used in a medical clinic laboratory, a use-dilution test or a disk-diffusion test?
  7-8

Chemical Methods of Microbial Control (2 of 2)

Learning Objectives

7-9 Identify the methods of action and preferred uses of chemical disinfectants.

7-10 Differentiate halogens used as antiseptics from halogens used as disinfectants.

7-11 Identify the appropriate uses for surface-active agents.

7-12 List the advantages of glutaraldehyde over other chemical disinfectants.

7-13 Identify chemical sterilizers.

Phenol and Phenolics

- Injure lipids of plasma membranes, causing leakage

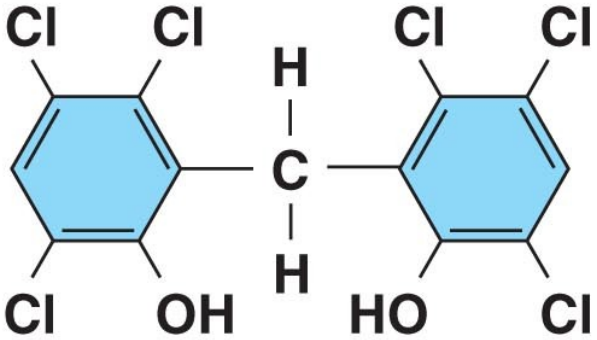
Figure 7.7a-b The Structure of Phenolics and Bisphenols

(a) Phenol **(b)** O-phenylphenol

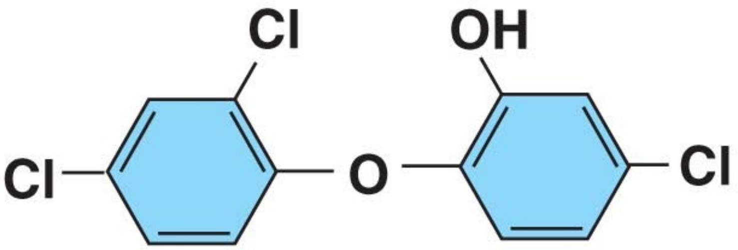
Bisphenols

- Contain two phenol groups connected by a bridge
- Hexachlorophene and triclosan
- Disrupt plasma membranes

Figure 7.7c-d The Structure of Phenolics and Bisphenols



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Biguanides

- Chlorhexidine
- Used in surgical hand scrubs
- Disrupt plasma membranes

Halogens

- Iodine
  - **Tincture:** solution in aqueous alcohol
  - **Iodophor:** combined with organic molecules
  - Impairs protein synthesis and alters membranes
- Chlorine
  - Oxidizing agents; shut down cellular enzyme systems
  - Bleach: hypochlorous acid (HOCl)
  - Chloramine: chlorine + ammonia

Alcohols (1 of 2)

- Denature proteins and dissolves lipids
- No effect on endospores and nonenveloped viruses
- Ethanol and isopropanol
  - Require water

Alcohols (2 of 2)

Table 7.6 Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution against Streptococcus pyogenes

	Time of Exposure				
Concentration of Ethanol (%)	**10**	**20**	**30**	**40**	**50**
100	G	G	G	G	G
95	NG	NG	NG	NG	NG
90	NG	NG	NG	NG	NG
80	NG	NG	NG	NG	NG
70	NG	NG	NG	NG	NG
60	NG	NG	NG	NG	NG
50	G	G	NG	NG	NG
40	G	G	G	G	G

Note:

G = growth

NG = no growth

Heavy Metals and Their Compounds

- **Oligodynamic action**—very small amounts exert antimicrobial activity
- Denature proteins
- Ag, Hg, Cu, Zn
  - Silver nitrate is used to prevent ophthalmia neonatorum
  - Mercuric chloride prevents mildew in paint
  - Copper sulfate is an algicide
  - Zinc chloride is found in mouthwash

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Figure 7.8 Oligodynamic Action of Heavy Metals

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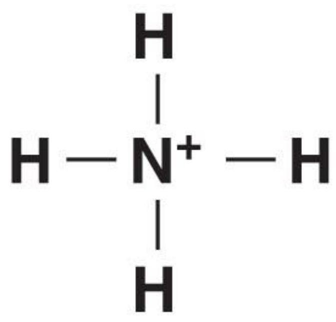
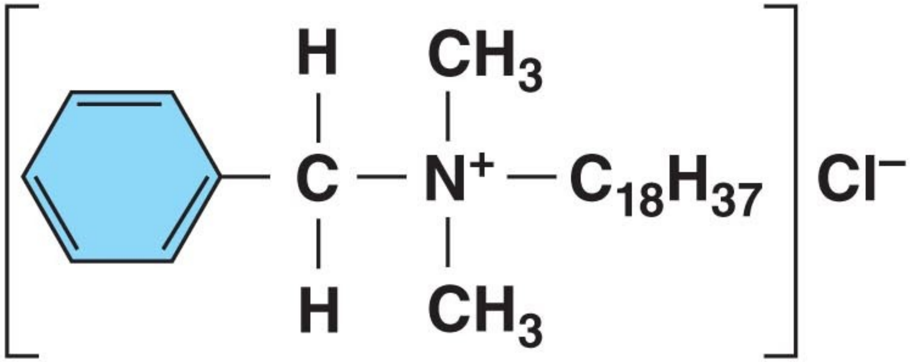
Surface-Active Agents

Soap	Degerming; emulsification
Acid-anionic sanitizers	Anions react with plasma membrane
Quaternary ammonium compounds (quats)	Cations are bactericidal, denature proteins, disrupt plasma membrane

Figure 7.9 The Ammonium Ion and a Quaternary Ammonium Compound, Benzalkonium Chloride (Zephiran)

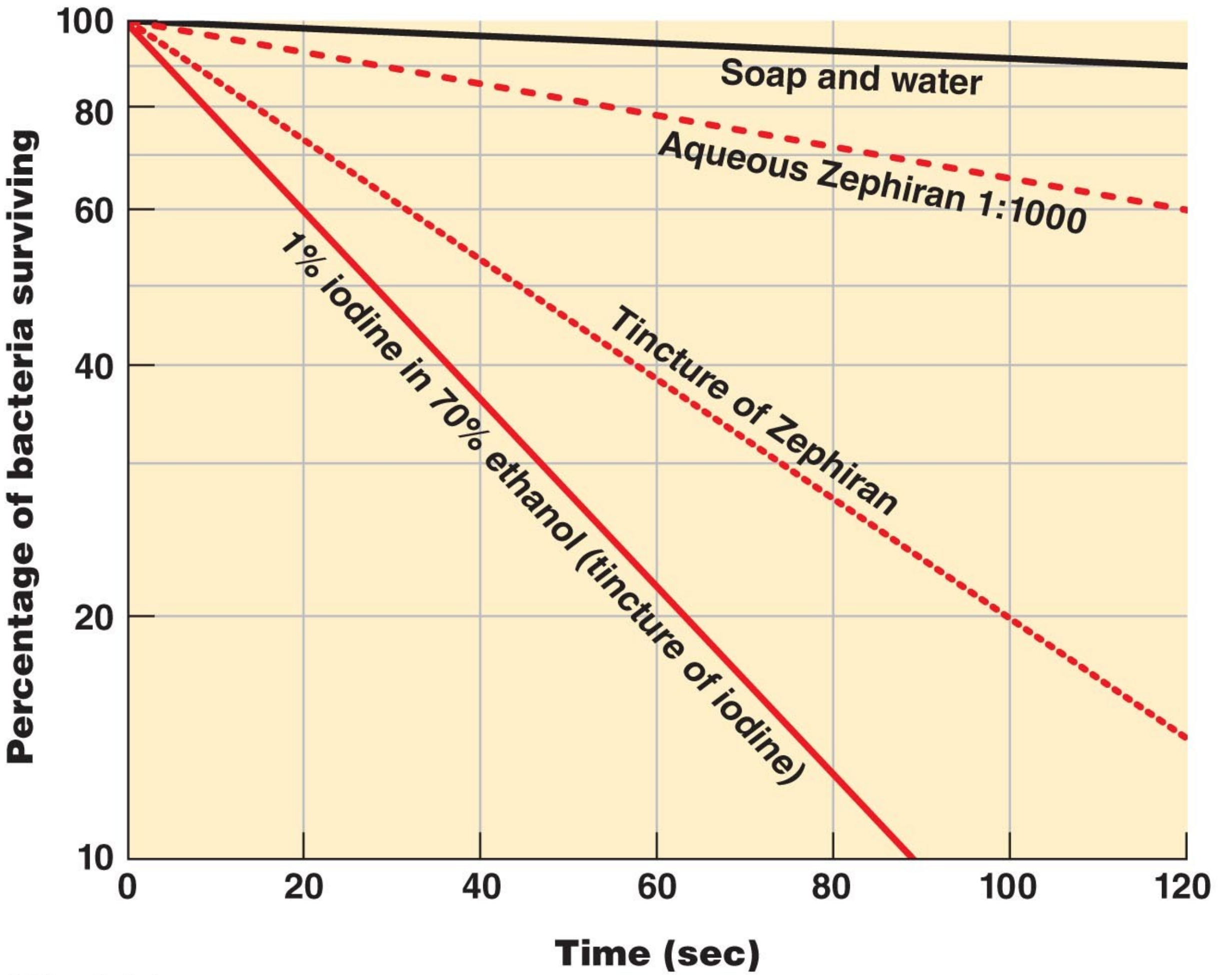


Ammonium ion



Benzalkonium chloride

Figure 7.10 A Comparison of the Effectiveness of Various Antiseptics



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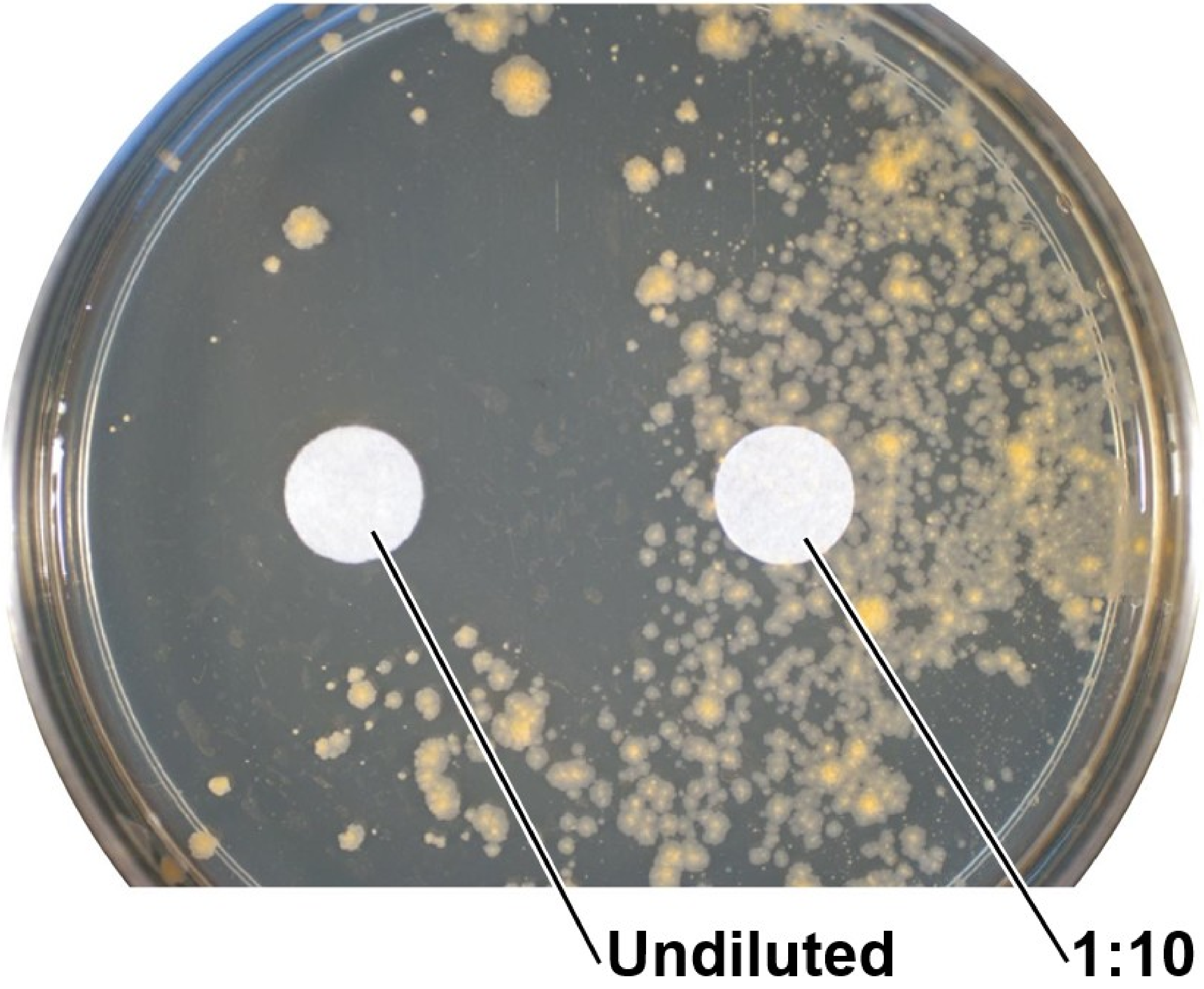
Clinical Focus: Infection Following Anesthesia Injection

- Which preparation is more effective?

Clinical Focus 7.1



Chemical Food Preservatives

- Sulfur dioxide prevents wine spoilage
- Organic acids
  - Inhibit metabolism
  - Sorbic acid, benzoic acid, and calcium propionate prevent molds in acidic foods
- Nitrites and nitrates prevent endospore germination

Antibiotics

- Bacteriocins—proteins produced by one bacterium that inhibits another
- Nisin and natamycin prevent spoilage of cheese

Aldehydes

- Inactivate proteins by cross-linking with functional groups $(-NH_2, -OH, -COOH, -SH)$
- Used for preserving specimens and in medical equipment
  – Formaldehyde and ortho-phthalaldehyde
  – Glutaraldehyde is one of the few liquid chemical sterilizing agents

Chemical Sterilization

- Gaseous sterilants cause alkylation—replacing hydrogen atoms of a chemical group with a free radical
- Cross-links nucleic acids and proteins
- Used for heat-sensitive material
  - Ethylene oxide

Plasma

- Fourth state of matter, consisting of electrically excited gas
- Free radicals destroy microbes
- Used for tubular instruments

Supercritical Fluids

- CO_2 with gaseous and liquid properties
- Used for medical implants

Peroxygens and Other Forms of Oxygen

- Oxidizing agents
- Used for contaminated surfaces and food packaging
  - O_3, H_2O_2, and peracetic acid

Check Your Understanding-6

Check Your Understanding

- ✓ Why is alcohol effective against some viruses and not others?
  7-9
- ✓ Is Betadine an antiseptic or a disinfectant when it is used on skin?
  7-10
- ✓ What characteristics make surface-active agents attractive to the dairy industry?
  7-11
- ✓ What chemical disinfectants can be considered sporicides?
  7-12
- ✓ What chemicals are used to sterilize?
  7-13

Microbial Characteristics and Microbial Control (1 of 2)

Learning Objective

7-14 Explain how the type of microbe affects the control of microbial growth.

Microbial Characteristics and Microbial Control (2 of 2)

Table 7.7 Effectiveness of Chemical Antimicrobials against Endospores and Mycobacteria

Chemical Agent	Effect against Endospores	Effect against Mycobacteria
Glutaraldehyde	Fair	Good
Chlorines	Fair	Fair
Alcohols	Poor	Good
Iodine	Poor	Good
Phenolics	Poor	Good
Chlorhexidine	None	Fair
Bisphenols	None	None
Quats	None	None
Silver	None	None

Check Your Understanding-7

Check Your Understanding

- ✓ The presence or absence of endospores has an obvious effect on microbial control, but why are gram-negative bacteria more resistant to chemical biocides than gram-positive bacteria? 7-14